

$$\mathbf{B} \leftarrow \begin{pmatrix} (\mathrm{Sia})_b \\ -\mathrm{GalNAc} - (\mathrm{Gal})_a - (\mathrm{Sia})_c - (\mathrm{R})_d \end{pmatrix}_e$$

a-c, e (independently selected) = 0 or 1; d = 0;  $R = modifying\ group,\ sialyl\ or \ oligosialyl$ 

FIG. 29A

# 61/498

CHO, BHK, 293 cells, Vero expressed G-CSF a-c, e (independently selected) = 0 or 1; d = 0

1. Sialidase
2. CMP-SA-PEG, ST3Gal1

a-d, e (independently selected) = 0 or 1;

R = PEG.

# FIG. 29B

Insect cell expressed G-CSF a, e (independently selected) = 0 or 1;

b, c, d = 0.

Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1; R = PEG.

FIG. 29C

```
E. coli expressed G-CSF
a-e = 0.
```

GalNAc Transferase, UDP-GalNAc
 CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

#### FIG. 29D

```
NSO expressed G-CSF
a, e (independently selected) = 0 or 1;
b, c, d = 0
```

```
1. CMP-SA-levulinate, ST3Gal1
2. H<sub>4</sub>N<sub>2</sub>-PEG
```

a, c, d, e (independently selected) = 0 or 1; b = 0; R = PEG.

#### FIG. 29E

# 63/498

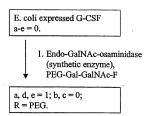


FIG. 29F

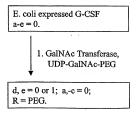
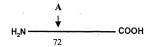


FIG. 29G



$$\begin{array}{c} \text{(Fuc)}_{i} \\ \text{GlcNAc-(Gal)}_{a}]_{e^{-}} \text{(Sia)}_{i}^{-} \text{(R)}_{v} \\ \text{[GlcNAc-(Gal)}_{b}]_{f^{-}} \text{(Sia)}_{k^{-}} \text{(R)}_{w} \\ \text{[GlcNAc-(Gal)}_{b}]_{g^{-}} \text{(Sia)}_{l^{-}} \text{(R)}_{x} \\ \text{Man} \\ \text{[[GlcNAc-(Gal)}_{d}]_{h^{-}} \text{(Sia)}_{m^{-}} \text{(R)}_{y} \\ \text{bb} \end{array}$$

a-d, i, n-u (independently selected) = 0 or 1. aa, bb, cc, dd, ee (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-z = 0; R = modifying group, mannose, oligo-mannose. R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 30A

```
GHO, BHK, 293 cells, Vero expressed interferon alpha 14C.
a-d, aa, bb = 1; e-h = 1 to 4; cc, j-m, i, r-u (independently selected) = 0 or 1; q, n-p, v-z, cc, dd, ee = 0.
```

Sialidase
 CMP-SA-PEG, ST3Gal3

```
a-d, aa, bb = 1; e-h = 1 to 4;
bb, cc, i, r-u (independently selected) = 0 or 1;
q, n-p, v-z, cc, dd, ee = 0;
v-y (independently selected) = 1,
when j-m (independently selected) = 1;
R = PEG.
```

#### FIG. 30B

```
Insect cell or fungi expressed interferon alpha-14C. a-d, f, h, j-q, s, u, v-z, cc, dd, ee = 0; e, g, i, r, t (independently selected) = 0 or 1; aa, bb = 1.
```

1. GNT's 1&2, UDP-GlcNAc 2. Galactosyltransferase, UDP-Gal-PEG

```
b, d, f, h, j-q, s, u, w, y, z, cc, dd, ee = 0;
a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1;
v, x (independently selected) = 1,
when a, c, (independently selected) = 1;
aa, bb = 1; R = PEG.
```

Yeast expressed interferon alpha-14C.
a-q, cc, dd, ee, v-z = 0;
r-y (independently selected) = 0 to 1;
aa, bb = 1;
R (branched or linear) = Man, oligomannose or polysaccharide.

Endo-H
 Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal3

a-z, bb=0; aa=1; R'=-Gal-Sia-PEG.

**FIG. 30D** 



$$(Fuc)_{i} \\ \mathbf{A} \leftarrow (Gal)_{a}l_{e} - (Sia)_{j} - (R)_{v} \\ - (Gic) \\ \mathbf{A} \leftarrow (Gic) \\ - (R^{2})_{d} \\ (R^{2})_{d} \\ (Gic) \\ \mathbf{A} \leftarrow (Gal)_{d}l_{f} - (Sia)_{e} - (R)_{w} \\ - (Gic) \\ \mathbf{A} \leftarrow (Gal)_{d}l_{f} - (Sia)_{f} - (R)_{w} \\ - (Gic) \\ \mathbf{A} \leftarrow (Gal)_{d}l_{f} - (Sia)_{f} - (R)_{w} \\ - (Gic) \\ \mathbf{A} \leftarrow (Gal)_{d}l_{f} - (Sia)_{f} - (R)_{w} \\ - (Gal)_{d}l_{f} - (Sia)_{f} - (R)_{g} \\ - (Gal)_{d}l_{f} - (R)_{g} \\ - (Gal)_{d}l_{f} - (R)_{g} \\ - (Gal)_{d}l_{f} - (R)_{g} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} \\ - (Gal)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)_{g}l_{f} - (R)$$

a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1. R = polymer; R' = sugar, glycoconjugate.

FIG. 30E

#### 68/498

```
CHO, BHK, 293 cells, Vero expressed interferon alpha-14C.

h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.
```

# 1. CMP-SA-PEG, ST3Gal3 ▼

```
h=1 to 3;
a-g, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
z=1; n=0; R=PEG.
```

#### FIG. 30F

```
Insect cell or fungi expressed interferon alpha-14C.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, r, t (independently selected) = 0 or 1;
z = 1.
```

- GNT's 1,2,4,5, UDP-GlcNAc
   Galactosyltransferase, UDP-Gal
- CMP-SA-PEG, ST3Gal3

```
a-m, r-y (independently selected) = 0 or 1;
z = 1; n = 0; R = PEG.
```

#### FIG. 30G

### 69/498

Yeast expressed interferon alpha-14C. a-n=0; r-y (independently selected) = 0 to 1; z=1; R (branched or linear) = Man, oligomannose.

```
1. mannosidases
```

- 2. GNT's 1,2,4,5, UDP-GlcNAc
- 3. Galactosyltransferase, UDP-Gal 4., CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

#### FIG. 30H

NSO expressed interferon alpha 14C. a-i, r-u (independently selected) = 0 or 1; j-m, n, v-y = 0; z = 1.

> CMP-SA-levulinate, ST3Gal3, buffer, salt
>  H<sub>4</sub>N<sub>2</sub>-PEG

a-i, j-m, r-y (independently selected) = 0 or 1; n = 0; z = 1; R = PEG.

FIG. 301

# 70/498

CHO, BHK, 293 cells, Vero expressed interferon alpha-14C.

h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.

1. CMP-SA-PEG, α2,8-ST

h = 1 to 3;
a-g, i, r-u (independently selected) = 0 or 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
z = 1; n = 0; R = PEG.

#### FIG. 30J

CHO, BHK, 293 cells, Vero expressed Interferon alpha-14C. a-g, j-m, r-u (independently selected) = 0 or 1; h = 1 to 3; n, v-y = 0; z = 1.

Sialidase
 Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1; h = 1 to 3; n = 0; z = 1; R = PEG.

#### FIG. 30K

# 71/498

```
\begin{array}{l} h=1 \text{ to } 3;\\ a\text{-g, i, r-u (independently selected)}=0 \text{ or } 1;\\ j\text{-m (independently selected)}=0 \text{ to } 40;\\ z=1; \text{ v-y, } n=0. \end{array}
```

#### FIG. 30L

```
Insect cell or fungi expressed interferon alpha-14C. a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.
```

```
    GNT's 1 & 2, UDP-GlcNAc
    Galactosyltransferase,
    UDP-Gal-linker-SA-CMP
    ST3Gal3, transferrin
```

```
a, c, e, g, i, r, t, v, x (independently selected) = 0 or 1; z = 1; b, d, f, h, j-n, s, u, w, y = 0; R = \text{transferrin}.
```

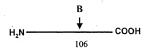
#### FIG. 30M

Insect cell or fungi expressed interferon alpha-14C. a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.

endoglycanase
 Galactosyltransferase,
 UDP-Gal-linker-SA-CMP
 ST3Gal3, transferrin

i (independently selected) = 0 or 1; a-h, j-m, r-z = 0; n = 1; R' = -Gal-linker-transferrin.

FIG. 30N



a-c, e, f (independently selected) = 0 or 1; d, g = 0; R = polymer, glycoconjugate.

FIG. 300

CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b). a-c (independently selected) = 0 or 1; e=1; d, f, g=0

1. Sialidase 2. CMP-SA-PEG, ST3Gal1

a-d (independently selected) = 0 or 1; e = 1; b, f, g = 0; R = PEG.

#### FIG. 30P

Insect cell expressed interferon alpha (2a or 2b). a, e (independently selected) = 0 or 1; b, c, d, f, g = 0.

Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal1

a, c, d, e (independently selected) = 0 or 1; b, f, g = 0; R = PEG.

FIG. 30Q

# 75/498

E. coli expressed IF-alpha (2a or 2b). a-g = 0.

GalNAc Transferase,
 UDP-GalNAc-PEG

a-c, f, g = 0; d, e = 1; R = PEG.

# FIG. 30R

NSO expressed IF-alpha (2a or 2b). a (independently selected) = 0 or 1;

e = 1; b, c, d, f, g = 0

1. CMP-SA-levulinate, ST3Gal1

2. H<sub>4</sub>N<sub>2</sub>-PEG

a, c, d (independently selected) = 0 or 1; e = 1; b, f, g = 0; R = PEG.

FIG. 30S

E. coli expressed IF-alpha (2a or 2b). a-g = 0.

 Endo-N-acetylgalatosamidase (synthetic enzyme), PEG-Gal-GalNAc-F

a, d, e = 1; b, c, f, g = 0; R = PEG.

### FIG. 30T

E. coli expressed IF-alpha (2a or 2b). a-g=0.

- 1. GalNAc Transferase, UDP-GalNAc
- 2. sialyltransferase, CMP-SA-PEG

b, d = 0 or 1; e = 1; a, c, f, g = 0; R = PEG.

FIG. 30U

# 77/498

```
CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b). a-c, f (independently selected) = 0 or 1; e=1; d, g=0
```

Sialidase

2. CMP-SA-PEG, ST3Gal1 and ST3Gal3

```
a-d, f, g (independently selected) = 0 or 1;
e = 1; R = PEG.
```

#### **FIG. 30V**

```
CHO, BHK, 293 cells, Vero expressed IF-alpha (2a or 2b). a-c, f (independently selected) = 0 or 1; e=1; d, g=0
```

1. Sialidase

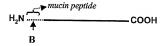
CMP-SA-linker-SA-CMP, ,ST3Gal1

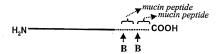
3. ST3Gal3, transferrin

a-d, f (independently selected) = 0 or 1; e = 1; R = transferrin; g = 0.

#### FIG. 30W







$$\mathbf{B} \quad \blacktriangleleft \begin{bmatrix} (\operatorname{Sia})_{b} \\ - (\operatorname{GalNAc-(Gal)}_{a} - (\operatorname{Sia})_{c} - (\operatorname{R})_{d} \end{bmatrix}_{c}$$

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer, glycoconjugate.

FIG. 30X

#### 79/498

CHO, BHK, 293 cells, Vero expressed interferon alpha-mucin fusion protein. a-c, e (independently selected) = 0 or 1; d = 0

- Sialidase
   CMP-SA-PEG, ST3Gal1
- a-d, e (independently selected) = 0 or 1; R = PEG.

# FIG. 30Y

Insect cell expressed interferon alpha-mucin fusion protein.

a, e (independently selected) = 0 or 1; b, c, d = 0.

Galactosyltransferase, UDP-Gal-PEG

a, d, e (independently selected) = 0 or 1; b, c = 0; R = PEG.

FIG. 30Z

# 80/498

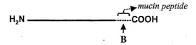
E. coli expressed interferon alpha-mucin fusion protein.

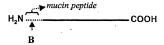
a-e = 0.

- 1. GalNAc Transferase, UDP-GalNAc
- 2. CMP-SA-PEG, sialyltransferase

c, d, e (independently selected) = 0 or 1; a, b = 0; R = PEG.

FIG. 30AA





$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{Sia})_{b} \\ - (\operatorname{GalNAc-(Gal)}_{a}-(\operatorname{Sia})_{c}-(R)_{d} \end{bmatrix}$$

$$C \leftarrow (R')_n$$

a-c, e (independently selected) = 0 or 1; d = 0; R = polymer, linker.

FIG. 30BB

E. coli expressed interferon alpha-mucin fusion protein.

a-e, n = 0.

 GalNAc Transferase, UDP-GalNAc-PEG

d, e (independently selected) = 0 or 1; a-c, n = 0; R = PEG.

#### FIG. 30CC

 $E.\ coli\ expressed\ \ interferon\ alpha-mucin\ fusion\ protein.$ 

a-e, n = 0.

- GalNAc Transferase,
   UDP-GalNAc-linker-SA-CMP
   ST3Gal3, asialo-transferrin
- 3. CMP-SA, ST3Gal3

d, e (independently selected) = 0 or 1; a-c, n = 0; R = linker-transferrin.

FIG. 30DD

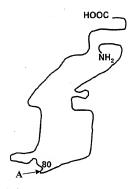
E. coli expressed Interferon alpha (no fusion). a-e, n = 0.

NHS-CO-linker-SA-CMP
 ST3Gal3, transferrin

a-e = 0; n = 1; R' = linker-transferrin.

FIG. 30EE

# 84/498



$$(Fuc)_{i} \\ \mathbf{A} \leftarrow \underbrace{\mathsf{GlcNAc}}_{i} \\ \mathsf{GlcNAc} - \mathsf{Gal}_{a} \\ \mathsf{GlcNAc} - \mathsf{Gal}_{b} \\ \mathsf{I}_{f} - \mathsf{Gia}_{h} \\ \mathsf{I}_{g} - \mathsf$$

a-d, i, r-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 or 1. n, v-y = 0; z = 0 or 1; R = polymer

FIG. 31A

### 85/498

CHO, BHK, 293 cells, Vero expressed IF-beta h=1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z = 1.

Sialidase
 CMP-SA-PEG, ST3Gal3

h=1 to 3; a-g, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; j-m, v-y (independently selected) = 0 or 1; z=1; n=0; R=PEG.

### FIG. 31B

Insect cell expressed IF-beta a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t (independently selected) = 0 or 1; z = 1.

 GNT's 1&2, UDP-GIcNAc
 Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal3, buffer, salt

 $\label{eq:continuous} \begin{array}{ll} b,\,d,\,f,\,h,\,k,\,m,\,n,\,s,\,u,\,w,\,y=0;\\ a,\,c,\,e,\,g,\,i,\,r,\,t\ \ (independently\ selected)=0\ \ or\ 1;\\ j,\,l,\,v,\,x\ \ (independently\ selected)=0\ \ or\ 1;\\ z=1;\ R=PEG. \end{array}$ 

#### FIG. 31C

```
Yeast expressed IF-beta
a-n = 0; z = 1;
r-y (independently selected) = 0 to 1;
R (branched or linear) = Man, oligomannose or polysaccharide.
```

```
1. Endo-H
```

2. Galactosyltransferase, UDP-Gal

↓ 3.. CMP-SA-PEG, ST3Gal3

a-m, r-z=0; n = 1; R' = -Gal-Sia-PEG.

### FIG. 31D

```
CHO, BHK, 293 cells, Vero expressed IF-beta h=1 to 3; a-g, j-m, i (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; n, v-y = 0; z=1.
```

1. CMP-SA-PEG, ST3Gal3

```
\begin{array}{l} h=1 \text{ to 3;} \\ a\text{-g, i (independently selected)} = 0 \text{ or 1;} \\ r\text{-u (independently selected)} = 0 \text{ or 1;} \\ j\text{-m, v-y (independently selected)} = 0 \text{ or 1;} \\ z=1; \text{ n=0; } R=PEG. \end{array}
```

#### FIG. 31F

### 87/498

$$\label{eq:continuous} \begin{split} &\text{Insect cell expressed IF-beta} \\ &\text{a-d, f, h, j-n, s, u, v-y = 0; e, g, i, r, t} \\ &\text{(independently selected) = 0 or 1; } z=1. \end{split}$$

GNT's 1,2,4,5, UDP-GleNAc
 Galactosyltransferase, UDP-Gal
 CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

#### FIG. 31F

Yeast expressed IF-beta a-n=0; z=1; r-y (independently selected) = 0 to 1; R (branched or linear) = Man, oligomannose.

- 1. mannosidases
- 2. GNT's 1,2,4,5, UDP-GICNAC
- 3. Galactosyltransferase, UDP-Gal
- 4.. CMP-SA-PEG, ST3Gal3

a-m, r-y (independently selected) = 0 or 1; z = 1; n = 0; R = PEG.

### 88/498

```
NSO expressed IF-beta
a-i, r-u (independently selected) = 0 or 1;
j-m, n, v-y = 0; z = 1.

1. CMP-SA-levulinate, ST3Gal3, buffer, salt
2. H_4N_2-PEG
a-i, j-m, r-y (independently selected) = 0 or 1;
n = 0; z = 1; R = PEG.
```

# FIG. 31H

```
CHO, BHK, 293 cells, Vero expressed IF-beta h = 1 to 3;
a-g, j-m, i (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
n, v-y = 0; z = 1.
```

1. CMP-SA-PEG, α2,8-ST

```
\begin{split} &h=1\ to\ 3;\\ &a\cdot g,\ i,\ r\cdot u\ (independently\ selected)=0\ or\ 1;\\ &j\cdot m\ (independently\ selected)=0\ to\ 2;\\ &v\cdot y\ (independently\ selected)=1,\\ &when\ j\cdot m\ (independently\ selected)\ is\ 2;\\ &z=1;\ n=0;\ R=PEG. \end{split}
```

#### FIG. 311

### 89/498

CHO, BHK, 293 cells, Vero expressed IF-beta a-g, j-m, r-u (independently selected) = 0 or 1; h=1 to 3; n, v-y=0; z=1.

- 1. Sialidase
- 2. Trans-sialidase, PEG-Sia-lactose

a-g, j-m, r-y (independently selected) = 0 or 1; h = 1 to 3; n = 0; z = 1; R = PEG.

#### FIG. 31J

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

- Sialidase
  - 2. CMP-SA-PEG (1.2 mol eq), ST3Gal3
  - 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n=0;

v-y (independently selected) = 0 or 1; R = PEG.

FIG. 31K

#### 90/498

```
NSO expressed Ifn-beta.
a-d, i-m, r-u, z (independently selected) = 0 or 1;
e-h = 1; n, v-y = 0;
Sia (independently selected) = Sia or Gal.
```

- Sialidase and α-galactosidase
   α-Galactosyltransferase, UDP-Gal
   3. CMP-SA-PEG, ST3Gal3
- a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; R = PEG n = 0; v-y (independently selected) = 1, when j-m (independently selected) is 1;

#### FIG. 31L

```
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n, v-y = 0.
```

- Sialidase
   CMP-SA-PEG (16 mol eq), ST3Gal3
   CMP-SA, ST3Gal3
- a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n = 0; v-y (independently selected) = 0 or 1; R = PEG.

# FIG. 31M

CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h = 1; n = 0; v-y (independently selected) = 0 or 1; R = PEG.

### FIG. 31N

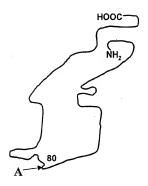
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, r-u, z (independently selected) = 0 or 1; e-h=1; n, v-y=0.

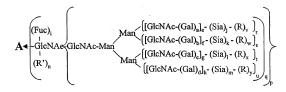
1. CMP-SA, α2,8-ST

a-d, i, r-u, z (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; n, v-y (independently selected) = 0.

FIG. 310

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a-d, i, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group; R' = H, glycosyl group, modifying group, glycoconjugate.

FIG. 31P

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```
Insect cell expressed Ifn-beta.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;

j-m=0; v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R=PEG.
```

#### FIG. 31Q

```
Yeast expressed Ifin-beta. 
a-m = 0; q-y (independently selected) = 0 to 1; p = 1; 
R (branched or linear) = Man, oligomannose.
```

- 1. Endoglycanase
  2. Galactosyltransferase, UDP-Gal

  3. CMP-SA-PEG, ST3Gal3
- a-m, p-y = 0; n (independently selected) = 0 or 1;

R' = -Gal-Sia-PEG.

#### FIG. 31R

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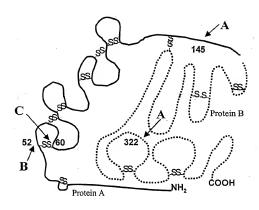
CHO, BHK, 293 cells, Vero expressed Ifn-beta. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.

- CMP-SA-linker-SA-CMP, ST3Gal3
   ST3Gal3, desialylated transferrin.
- 3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-transferrin.

FIG. 31S

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$$\mathbf{A} \leftarrow \begin{bmatrix} [\operatorname{GlcNAc-(Gal)_a}]_e^* \cdot (\operatorname{Sia})_j - (R)_v \\ | [\operatorname{GlcNAc-(Gal)_b}]_t^* \cdot (\operatorname{Sia})_k - (R)_w \\ | [\operatorname{GlcNAc-(Gal)_b}]_t^* \cdot (\operatorname{Sia})_k - (R)_w \\ | [\operatorname{GlcNAc-(Gal)_b}]_g^* \cdot (\operatorname{Sia})_j - (R)_x \\ | [\operatorname{GlcNAc-(Gal)_d}]_h^* \cdot (\operatorname{Sia})_m^* - (R)_y \\ | [\operatorname{GlcNAc-(Gal)_d}]_h^* \cdot (\operatorname{Sia})_m^* \cdot (R)_y \\ | [\operatorname{GlcNAc-(Gal)_d}]_h^* \cdot (R)_y \\ | [\operatorname{GlcNAc-(Gal)_d}]_h^*$$

 $\mathbf{B} \leftarrow \left( \text{Glc-}(\mathbf{X}\mathbf{y}\mathbf{l})_{n} \right)_{0}$ 

**C ←**[-Fuc]<sub>n</sub>

a-d, i, q-u (independently selected) = 0 or 1. o, p (independently selected) = 0 or 1. e-h, n (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-y = 0;

R = modifying group, mannose, oligomannose, Sia-Lewis X, Sia-Lewis A...

FIG. 32A

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```
BHK expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.
```

```
    Sialidase
    CMP-SA-PEG (16 mole eq),
ST3Gal3
```

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4; v, x, (independently selected) = 1, when j, l (respectively, independently selected) is 1; R = PEG.
```

#### FIG. 32B

CHO, BHK, 293 cells, Veto expressed Factor VII or VIIa a-d, e, i, g, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, v-y = 0; n = 0-4.

```
1. Sialidase
2. CMP-SA-PEG (1.2 mole eq),
ST3Gal3
3. CMP-SA (8 mol eq), ST3Gal3
```

```
a-d, e, g, i, q, j, l, o, p (independently selected) = 0 or 1; r, t = 1; f, h, k, m, s, u, w, y = 0; n = 0-4; v or x, (independently selected) = 1, when j or l, (respectively, independently selected) is 1; R = PEG.
```

#### FIG. 32C

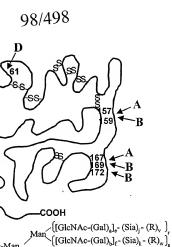
# 97/498

NSO expressed Factor VII or VIIa a--u (independently selected) = 0 or 1; v-y = 0; n = 0-4; Sia (independently selected) = Sia or Gal.

- 1. Sialidase and α-galactosidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, o-u (independently selected) = 0 or 1; n = 0.4; v-y (independently selected) = 1, when j-m (independently selected) is 1; Sia = Sia; R = PEG.

FIG. 32D



$$\mathbf{A} \leftarrow \text{-GlcNAc-GlcNAc-Man} \begin{bmatrix} [\text{GlcNAc-(Gal)}_b]_r \cdot (\text{Sia})_k \cdot (R)_w \end{bmatrix}_s \\ \text{Man} \begin{bmatrix} [\text{GlcNAc-(Gal)}_a]_g \cdot (\text{Sia})_l \cdot (R)_y \end{bmatrix}_t \\ [(\text{GlcNAc-(Gal)}_a]_h \cdot (\text{Sia})_m \cdot (R)_y \end{bmatrix}_u \\ \mathbf{B} \leftarrow \begin{bmatrix} (\text{Sia})_o \cdot (R)_g \end{bmatrix} \quad \mathbf{C} \leftarrow \begin{bmatrix} (\text{Glc-(Xyl)}_{ab})_{ab} \cdot (R)_g \end{bmatrix}_t \\ \mathbf{C} \leftarrow \begin{bmatrix} (\text{Glc-(Xyl)}_{ab})_{ab} \cdot (R)_g \cdot (R)_g \end{bmatrix}_t \\ \mathbf{C} \leftarrow \begin{bmatrix} (\text{Glc-(Xyl)}_{ab})_{ab} \cdot (R)_g \cdot (R)_$$

$$\mathbf{D} \leftarrow -\text{Fuc}\left\{-(\text{GlcNAc})_{\text{cc}} - (\text{Gal})_{\text{dd}} - (\text{Sia})_{\text{ee}}\right\} - (R)_{\text{gg}}$$

(Fuc).

a-d, i, n-u (independently selected) = 0 or 1. bb, cc, dd, ec, ff, gg (independently selected) = 0 or 1. e-h, aa (independently selected) = 0 to 6. j-m (independently selected) = 0 to 20. v-z = 0: R = modifying group, mannose, oligo-mannose,

**FIG. 33A** 

# 99/498

CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

Sialidase

2. CMP-SA-PEG, ST3Gal3

#### FIG. 33B

CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

1. Sialidase

2. CMP-SA-PEG, ST3Gal3

3. ST3Gal1, CMP-SA

a-d, n, p, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, i, r-u (independently selected) = 0 or 1; j-m, ee, v-y, gg (independently selected) = 0 or 1; o, z = 0: R = PEG.

#### FIG. 33C

CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; ee, j-m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

```
    sialidase
```

- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
  4. CMP-SA-PEG, ST3Gal1

```
a-d, n, q = 1; e-h = 1 to 4;
aa, bb, co, dd, ee, ff, i, r-u (independently selected) = 0 or 1; R = PEG;
o, v-y, gg = 0;
j-m, p, ee (independently selected) = 0 or 1, but when p = 1, z = 1.
```

#### FIG. 33D

```
CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q=1; e-h=1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg=0.
```

CMP-SA-PEG, ST3Gal3

```
a-d, q = 1; e-h = 1 to 4;
aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected) = 0 or 1; R = PEG;
o, p, z = 0; j-m, ee, v-y, gg (independently selected) = 0 or 1.
```

#### FIG. 33E

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CHO, BHK, 293 cells, Vero expressed Factor IX a-d, q = 1; e-h = 1 to 4; aa, bb, cc, dd, ee, ff, j-m, i, n, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

1. CMP-SA-levulinate, ST3Gal3, buffer, salt

▼ 2. H<sub>4</sub>N<sub>2</sub>-PEG

```
a-d, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)

= 0 or 1;

o, p, z = 0; R = PEG;

j-m, ee, v-y, gg (independently selected) = 0 or 1.
```

#### FIG. 33F

CHO, BHK, 293 cells, Vero expressed Factor IX a-d, n, q, bb, cc, dd, ff = 1; e-h, aa = 1 to 4; e-e, j.m, i, o, p, r-u (independently selected) = 0 or 1; v-z, gg = 0.

1. CMP-SA-PEG,  $\alpha$ 2,8-ST

```
a-d, q = 1; e-h = 1 to 4;

aa, bb, cc, dd, ee, ff, i, n, r-u (independently selected)

= 0 or 1;

o, p, z = 0; R= PEG;

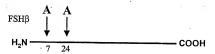
j-m, ee (independently selected) = 0 to 2;

v-y, gg (independently selected) = 1, when j-m

(independently selected) is 2;
```

#### FIG. 33G





$$\mathbf{A} \bullet \begin{array}{c} \text{(Fuc)}_{i} \\ \text{GlcNAc-GlcNAc-Man} \\ \text{Man} \underbrace{ \begin{array}{c} [[GlcNAc-(Gal)_{a}]_{c}^{-} (Sia)_{j} - (R)_{v} \end{array} \right]_{r}^{r}}_{r} \\ \text{([GlcNAc-(Gal)_{b}]_{r}^{-} (Sia)_{k}^{-} - (R)_{w}]_{s}^{r}} \\ \text{Man} \underbrace{ \begin{array}{c} [[GlcNAc-(Gal)_{d}]_{c}^{-} (Sia)_{j} - (R)_{v} \end{array} \right]_{r}^{r}}_{r} \\ \text{([GlcNAc-(Gal)_{d}]_{r}^{-} (Sia)_{j}^{-} - (R)_{v} \end{array} \right]_{r}^{r}}_{r} \\ \text{([GlcNAc-(Gal)_{d}]_{h}^{-} (Sia)_{m}^{-} - (R)_{v} \end{array} )_{r}^{r}}_{r}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose.

**FIG. 34A** 

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- 1. Sialidase
- 2. CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

# FIG. 34B

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.

- 1. Sialidase
  - CMP-SA-PEG (1.2 mol eq), ST3Gal3
  - 3. CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 34C

NSO expressed FSH.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.

- Sialidase and α-galactosidase
   Galactosyltransferase, UDP-Gal
- **★** 3. CMP-SA-PEG, ST3Gal3

 $\begin{array}{lll} a\text{-d}, i\text{-m}, q\text{-u} \ (independently \ selected) = 0 \ or \ 1; \\ e\text{-h} = 1; \ v\text{-y} \ (independently \ selected) = 1, \\ when j\text{-m} \ (independently \ selected) \ is \ 1; \\ R = PEG. \end{array}$ 

#### FIG. 34D

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.

- Sialidase
   CMP-SA-PEG (16 mol eq),
- ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

#### FIG. 34E

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CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H<sub>x</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

## FIG. 34F

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 34G

$$\begin{split} &\text{Insect cell expressed FSH.} \\ &\text{a-d, f, h, j-m, s, u, v-y} = 0; \\ &\text{e, g, i, q, r, } \ t \ (&\text{independently selected}) = 0 \ \text{or} \ 1. \end{split}$$

GNT's 1,2,4,5, UDP-GlcNAc
 Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1; j-m = 0; v-y (independently selected) = 1, when e-h (independently selected) is 1; R = PEG.

## FIG. 34H

Yeast expressed FSH. a-m = 0; q-y (independently selected) = 0 to 1; p = 1; R (branched or linear) = Man, oligomannose.

1. Endoglycanase
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

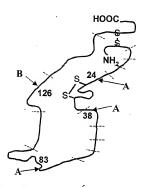
#### FIG. 341

CHO, BHK, 293 cells, Vero expressed FSH. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

- CMP-SA-linker-SA-CMP, ST3Gal3
   ST3Gal1, desialylated chorionic gonadrophin (CG) produced in CHO.
   CMP-SA, ST3Gal3, ST3Gal1
- a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-CG.

FIG. 34J

## 108/498



$$\begin{array}{c} (\operatorname{Fuc})_{i} \\ \mathbf{A} & \leftarrow -\operatorname{GlcNAc-GlcNAc-Man} \\ & \left[ (\operatorname{GlcNAc-Gal})_{a} \right]_{e}^{-} (\operatorname{Sia})_{p}^{-} (R)_{v} \right]_{r} \\ & \left[ (\operatorname{GlcNAc-Gal})_{a} \right]_{e}^{-} (\operatorname{Sia})_{r}^{-} (R)_{v} \right]_{s} \\ & \left[ (\operatorname{GlcNAc-Gal})_{d} \right]_{e}^{-} (\operatorname{Sia})_{r}^{-} (R)_{v} \right]_{u} \\ & \mathbf{B} & \left( -\operatorname{Gal})_{A} \right]_{e}^{-} (\operatorname{Sia})_{p}^{-} (R)_{z} \\ & \left[ (\operatorname{GlcNAc-(Gal)})_{d} \right]_{h}^{-} (\operatorname{Sia})_{m}^{-} (R)_{y} \right]_{u} \\ \end{array}$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z=0; R=polymer.

FIG. 35A

## 109/498

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to1; v-z=0
```

2. CMP-SA-PEG, ST3Gal3

1. Sialidase

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 or 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

### FIG. 35B

```
Insect cell expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.
```

1. GNT's 1&2, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal
2. CMP-SA-PEG, ST3Gal3

 $\begin{array}{l} b,\,d,\,f,\,h,\,k,\,m\hbox{-}q,\,s,\,u,\,w,\,y,\,z=0;\\ a,\,c,\,e,\,g,\,i,\,r,\,t\ \ (independently\ selected)=0\ or\ 1;\\ j,\,l,\,v,\,x\,(independently\ selected)=0\ or\ 1;\\ R=PEG. \end{array}$ 

#### FIG. 35C

# 110/498

CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z=0.

- sialidase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA, ST3Gal3
- ↓ 4. CMP-SA-PEG, ST3Gal1

```
a-h, n, q = 1;
i-m, o, r-u (independently selected) = 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

### FIG. 35D

```
CHO, BHK, 293 cells, Vero expressed EPO a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z = 0
```

1. CMP-SA-PEG, ST3Gal3

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m, v-y (independently selected) = 0 or 1;
R = PEG; z = 0.
```

#### FIG. 35E

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

GNT's 1, 2 & 5, UDP-GlcNAc
 Galactosyltransferase, UDP-Gal-PEG

a-c, e-g, i, i-t, v-x (independently selected) = 0 or 1; d, h, i-q, u, y, z = 0; R = PEG.

## FIG. 35F

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- GNT's 1, 2 & 5, UDP-GlcNAc
   Galactosidase (synthetic enzyme),
   PEG-Gal-F
- a-c, e-g, n, q-t, v-x, z (independently selected) = 0 or 1; d, h, j-m, o, p, y, z = 0; R = PEG.

FIG. 35G

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z=0; e, g, i, r, t (independently selected) = 0 or 1.

1. GNT-1, UDP-GlcNAc-PEG

e, i, r, v (independently selected) = 0 or 1; a-h, j-q, s-u, w-z = 0; R = PEG.

## FIG. 35H

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z=0; e, g, i, r, t (independently selected) = 0 or 1.

- GNT-1, UDP-GlcNAc
  - 2. Galactosyltransferase, UDP-Gal-PEG

a, e, i, r, v (independently selected) = 0 or 1; b-d, f-h, i-q, s-u, w-z = 0: R = PEG.

#### FIG. 351

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- GNT-1, UDP-GlcNAc
   Galactosyltransferase, UDP-Gal
- ▼ 3. ST3Gal3, CMP-SA-PEG

a, e, i, j, r, v (independently selected) = 0 or 1; b-d, f-h, k-q, s-u, w-z = 0; R = PEG.

## FIG. 35J

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- 1. GNT's 1, 2 & 5, UDP-GlcNAc
- Galactosyltransferase, UDP-Gal
- 3. ST3Gal3, CMP-SA-PEG

a-c, e-g, i-l, r-t, v-x (independently selected) = 0 or 1; d, h, m-q, u, y, z = 0; R = PEG.

#### FIG. 35K

Insect cell, yeast or fungi expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1.

- GNT's 1, 2 & 5, UDP-GlcNAc
   Galactosyltransferase, UDP-Gal
- ▼ 3. α2,6-sialyltransferase, CMP-SA-PEG

```
 \begin{array}{l} a\text{-c, e-g, i-l, r-t, v-x (independently selected)} \\ = 0 \text{ or l;} \\ d, h, m\text{-q, u, y, z} = 0; \quad R = PEG. \end{array}
```

## FIG. 35L

```
CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
v-z = 0.

1. sialidase
2. CMP-SA, ST3Gal3
3. CMP-SA-PEG, ST3Gal1

a-h, q, i-o, r-u (independently selected)
= 0 or 1;
v-y = 0; p, z = 0 or 1; R = PEG.
```

#### FIG. 35M

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```
CHO, BHK, 293 cells, Vero expressed EPO
a-q, r-u (independently selected) = 0 or 1;
v-z = 0.

1. CMP-SA-PEG, ST3Gal3
```

a-h, i-o, q-u (independently selected) = 0 or 1; v-y = 0; p, z = 0 or 1; R = PEG.

## FIG. 35N

CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

1. CMP-SA-PEG, α2,8-sialyltransferase

a-h, i-o, q-u (independently selected) = 0 or 1; v-y = 0; p, z = 0 or 1; R = SA-PEG.

FIG. 350

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CHO, BHK, 293 cells, Vero expressed EPO a-q, r-u (independently selected) = 0 or 1; v-z = 0.

CMP-SA-PEG, α2,8-sialyltransferase

a-h, i-o, p-u, v-z (independently selected) = 0 or 1; R = SA-PEG.

## FIG. 35P

yeast or fungi expressed EPO r, t, u, v, x, y (independently selected) = 0 or 1; a-m, n-q, s, w, z = 0;  $R = (Man)_n$  where n = 1.5, linear or branched.

mannosidases
 GNT-1, UDP-GlcNAc
 galactosyltransferase, UDP-Gal
 ST3Gal3, CMP-SA-PEG

a, e, j, r, v (independently selected) = 0 or 1; b-d, f-i, k-q, s-u, w-z = 0; R = PEG.

FIG. 35Q

# 117/498

yeast or fungi expressed EPO r, t, u, v, x, y (independently selected) = 0 or 1; a-m, n-q, s, w, z = 0;  $R = (Man)_n$  where n = 1-5, linear or branched.

mannosidases
 GNT-1, UDP-GlcNAc-PEG

e, r, v (independently selected) = 0 or 1; a-h, i-q, s-u, w-z = 0; R = PEG.

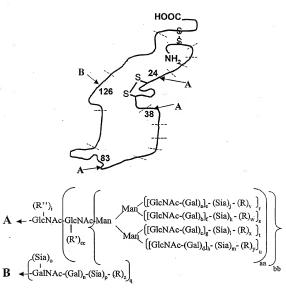
### FIG. 35R

yeast or fungi expressed EPO r, t, u, v, x, y (independently selected) = 0 or 1; a-m, n-q, s, w, z = 0;  $R = (Man)_n$  where n = 1-5, linear or branched.

- mannosidase-I
   GNT-1, UDP-GlcNAc
   galactosyltransferase, UDP-Gal
- 4. ST3Gal3, CMP-SA-PEG

a, e, j, r, t-u, v, x, y (independently selected) = 0 or 1; b-d, f-i, k-q, s, w, z = 0;  $(R)_v = PEG$ ;  $(R)_x$  and  $(R)_v = Man$ .

### FIG. 35S



a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

v-z=0; aa, bb=1; cc=0;

R = polymer; R" and R' = sugar-polymer or Fuc.

FIG. 35T

```
yeast or fungi expressed EPO r, t, u, v, x, y (independently selected) = 0 or 1; cc, a-m, n-q, s, w, z = 0; aa, bb = 1; R = (Man), where n = 1-100, linear or branched.
```

```
    1. endo-H
    2. galactosyltransferase, UDP-Gal-PEG
```

```
i (independently selected) = 0 or 1;
aa, bb, cc, a-h, j-z = 0; R" = Gal-PEG.
```

#### FIG. 35U

```
yeast or fungi expressed EPO r, t, u, v, x, y (independently selected) = 0 or 1; cc, a-m, n-q, s, w, z = 0; aa, bb = 1; R = (Man)_n where n = 1-100, linear or branched.
```

- endo-H
   galactosyltransferase, UDP-Gal
- 3. ST3Gal3, CMP-SA-PEG

i (independently selected) = 0 or 1; aa, bb, cc, a-h, j-z = 0; R" = Gal-SA-PEG.

### FIG. 35V

## 120/498

```
Insect cell expressed EPO a-d, f, h, j-m, n-q, s, u, v-z = 0; e, g, i, r, t (independently selected) = 0 or 1; aa = 1; \mathbb{R}^{2} = Fuc.
```

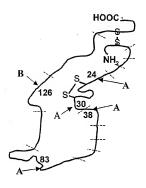
mannosidases

2. galactosyltransferase, UDP-Gal-PEG

cc, e, i, r, v (independently selected) = 0 or 1; bb, a-h, j-q, s-u, w-z = 0; aa = 1; R' = Gal-PEG.

FIG. 35W

# 121/498



$$\begin{array}{c} (\operatorname{Fuc})_{i} \\ \mathbf{A} \leftarrow -\operatorname{GlcNAc-GlcNAc-Man} \\ & \begin{array}{c} (\operatorname{GlcNAc-(Gal)}_{a}]_{e} \cdot (\operatorname{Sia})_{j} \cdot (R)_{v} \\ [\operatorname{GlcNAc-(Gal)}_{b}]_{f} \cdot (\operatorname{Sia})_{k} \cdot (R)_{w} \\ [\operatorname{GlcNAc-(Gal)}_{c}]_{g} \cdot (\operatorname{Sia})_{j} \cdot (R)_{x} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (\operatorname{Sia})_{m} \cdot (R)_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (\operatorname{Gal})_{m} \cdot (R)_{y} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (R)_{m} \cdot (R)_{m} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (R)_{m} \cdot (R)_{m} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (R)_{m} \cdot (R)_{m} \cdot (R)_{m} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (R)_{m} \cdot (R)_{m} \cdot (R)_{m} \cdot (R)_{m} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (R)_{m} \cdot (R)_{m} \cdot (R)_{m} \cdot (R)_{m} \\ [\operatorname{GlcNAc-(Gal)}_{d}]_{h} \cdot (R)_{m} \\ [\operatorname{GlcN$$

i-m (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. v-z = 0; R = polymer.

FIG. 35X

## 122/498

```
NSO expressed NESP q = 1; a-i, n, r-u (independently selected) = 0 or 1; j-m, o, p, v-z = 0
```

```
1. CMP-SA-levulinate, ST3Gal3,
buffer, salt
▼ 2. H<sub>4</sub>N<sub>2</sub>-PEG
```

```
q = 1; a-i, j-n, r-y (independently selected) = 0 or 1;
o, p. z = 0; R = PEG.
```

## FIG. 35Y

```
CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 or 1; v-z=0
```

1. CMP-SA-PEG, α2,8-ST

```
a-g, n, q = 1; h = 1 to 3;
i, o, p (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1;
j-m (independently selected) = 0 to 2;
v-y (independently selected) = 1,
when j-m (independently selected) is 2;
R = PEG; z = 0.
```

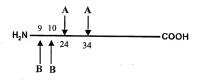
#### FIG. 35Z

CHO, BHK, 293 cells, Vero expressed NESP a-g, n, q = 1; h = 1 to 3; j-m, i, o, p (independently selected) = 0 or 1; r-u (independently selected) = 0 to 1; v-z = 0

 $1\,\text{CMP-SA},\,\text{poly-}\alpha2,\!8\text{-ST}$ 

a-g, n, q = 1; h = 1 to 3; i, j-m, o, p, r-u, (independently selected) = 0 or 1; v-z (independently selected) = 0-40; R = Sia.

FIG. 35AA



$$\mathbf{A} \leftarrow - \underbrace{ \begin{aligned} & (\operatorname{Fuc})_i \\ & -\operatorname{GlcNAc-GlcNAc-Man} \end{aligned} \underbrace{ \begin{aligned} & \underset{\left[[\operatorname{GlcNAc-(Gal)}_{a}]_e^- \left(\operatorname{Sia}\right)_i^- \left(\operatorname{R}\right)_v \right]_r}{\operatorname{Man} \left[[\operatorname{GlcNAc-(Gal)}_{b}]_r^- \left(\operatorname{Sia}\right)_i^- \left(\operatorname{R}\right)_w \right]_s} \\ & \underset{\left[[\operatorname{GlcNAc-(Gal)}_{d}]_h^- \left(\operatorname{Sia}\right)_m^- \left(\operatorname{R}\right)_v \right]_u}{\operatorname{Man} \left[[\operatorname{GlcNAc-(Gal)}_{d}]_h^- \left(\operatorname{Sia}\right)_m^- \left(\operatorname{R}\right)_v \right]_u} \end{aligned}}$$

$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{Sia})_{o} \\ -(\operatorname{GalNAc-(Gal})_{n}-(\operatorname{Sia})_{p}-(R)_{g_{ac}} \end{bmatrix}$$

a-d, i, n-u, aa (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer, glycoconjugate.

FIG. 36A

```
CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h=1; v-z=0.
```

 Sialidase
 CMP-SA-PEG (16 mol eq), ST3Gal3

```
a-d, i-m, q-u, aa (independently selected) = 0 or 1;
o, p, z = 0; n, e-h = 1;
v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

### FIG. 36B

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

- Sialidase
- 2. CMP-SA-PEG (1.2 mol eq),
- ST3Gal3 3. CMP-SA (16 mol eq), ST3Gal3 &
- ST3Gal1

a-d, i-m, p-u, as (independently selected) = 0 or 1; o, z = 0; n, e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

#### FIG. 36C

```
NSO expressed GM-CSF.
a-d, i-m, o-u, aa (independently selected) = 0 or 1;
n, e-h = 1; v-z=0;
Sia (independently selected) = Sia or Gal.
```

- Sialidase and α-galactosidase
   CMP-SA, ST3Gal3
- 2. CMP-SA, ST3Gal3
  2. CMP-SA-PEG, ST3Gal1
- i-m n-11 7 22 (independently delected) = 0 ---

a-d, i-m, p-u, z, aa (independently selected) = 0 or 1; n, e-h = 1; o, v-y = 0; z = 1, when p = 1; R = PEG.

#### FIG. 36D

```
CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.
```

- 1. Sialidase
- CMP-SA-PEG (16 mol eq), ST3Gal3
- 3. CMP-SA, ST3Gal3

a-d, i-m, q-y, aa (independently selected) = 0 or 1; o, p, z = 0; n, e-h = 1; R = PEG.

#### FIG. 36E

CHO, BHK, 293 cells, Vero expressed GM-CSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z = 0.

 CMP-SA-levulinate, ST3Gal3, buffer, salt
 H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, o-y, aa (independently selected) = 0 or 1; z = 0; n, e-h = 1; R = PEG.

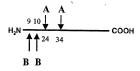
#### FIG. 36F

CHO, BHK, 293 cells, Vero expressed GMCSF. a-d, i-m, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; v-z=0.

1. CMP-SA, α2,8-ST

a-d, i, o-u, aa (independently selected) = 0 or 1; n, e-h = 1; j-m (independently selected) = 0-20; v-z (independently selected) = 0.

FIG. 36G



$$A \leftarrow \begin{array}{c} \text{(Fuc)}_{i} \\ \text{GlcNAc-(Gal)}_{a}l_{c} \text{- (Sia)}_{j} \text{- (R)}_{v} \end{array} \right]_{t} \\ \text{(R')}_{cc} \\ \text{(BlcNAc-(Gal)}_{b}l_{f} \text{- (Sia)}_{h} \text{- (R)}_{w} \\ \text{(R')}_{cc} \\ \text{(GlcNAc-(Gal)}_{d}l_{h} \text{- (Sia)}_{m} \text{- (R)}_{y} \\ \text{(R')}_{bb} \\ \text{(GlcNAc-(Gal)}_{d}l_{h} \text{- (Sia)}_{m} \text{- (R)}_{y} \\ \text{(R')}_{bb} \\ \text{(GlcNAc-(Gal)}_{d}l_{h} \text{- (Sia)}_{m} \text{- (R)}_{y} \\ \text{(R')}_{bb} \\ \text{(R')}_{bc} \\ \text{(R')}_{bc}$$

$$\mathbf{B} \leftarrow \begin{bmatrix} (\operatorname{Sia})_{o} \\ -\operatorname{GalNAc-(Gal)}_{n} - (\operatorname{Sia})_{p} - (R)_{z} \end{bmatrix}_{na}$$

a-d, i, n-u, aa, bb, cc (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y = 0; R = modifying group, mannose, oligo-mannose.

R'= H, glycosyl residue, modifying group. glycoconjugate.

FIG. 36H

# 129/498

```
Insect cell expressed GM-CSF. a-d, f, h, j-m, o, p, s, u, v-z = 0; e, g, i, n, q, r, t, aa (independently selected) = 0 or 1.
```

GNT's 1,2,4,5, UDP-GlcNAc
 Galactosyltransferase, UDP-Gal-PEG

```
a-i, n, q-u (independently selected) = 0 or 1;
j-m = 0; v-y (independently selected) = 1,
when e-h (independently selected) is 1;
R = PEG.
```

#### FIG. 361

```
Yeast expressed GM-CSF.
a-p, z, cc = 0;
q-y, aa (independently selected) = 0 to 1;
bb = 1; R (branched or linear) = Man, oligomannose;
GalNAc = Man.
```

```
    Endoglycanase
    mannosidase (if aa = 1).
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-p, r-z, aa, bb = 0;
q, cc (independently selected) = 0 or 1;
R' = -Gal-PEG.
```

#### FIG. 36J

CHO, BHK, 293 cells, Vero expressed GM-CSF. a--m, o-u, aa, bb (independently selected) = 0 or 1; n, v-z, cc = 0.

- 1. sialidase
- 2. CMP-SA, ST3Gal3
- 2. CMP-SA-linker-SA-CMP, ST3Gal1
- 3. ST3Gal3, transferrin

a--m, p-u, z, as (independently selected) = 0 or 1; o, v-y, cc = 0; bb, n = 1; R = transferrin.

FIG. 36K



$$\mathbf{A} \leftarrow \begin{bmatrix} \operatorname{Fuc})_{i} & \operatorname{Man} & \left[ [\operatorname{GlcNAc-(Gal)_{a}]_{e^{-}}} (\operatorname{Sia})_{j^{-}} (\operatorname{R})_{v} \right]_{r} \\ \operatorname{GlcNAc-GlcNAc-Man} & \operatorname{Man} & \left[ [\operatorname{GlcNAc-(Gal)_{b}]_{f^{-}}} (\operatorname{Sia})_{i^{-}} (\operatorname{R})_{w} \right]_{s} \\ \operatorname{Man} & \left[ [\operatorname{GlcNAc-(Gal)_{d}]_{g^{-}}} (\operatorname{Sia})_{i^{-}} (\operatorname{R})_{x} \right]_{t} \\ \left[ [\operatorname{GlcNAc-(Gal)_{d}]_{h^{-}}} (\operatorname{Sia})_{m^{-}} (\operatorname{R})_{y} \right]_{u} \\ \end{bmatrix}_{q} \end{aligned}$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 37A

# 132/498

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
1. Sialidase
2. CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

#### FIG. 37B

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (1.2 mol eq),
```

3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

ST3Gal3

### FIG. 37C

## 133/498

```
NSO expressed Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0;
Sia (independently selected) = Sia or Gal.
```

- Sialidase and α-galactosidase
   α-Galactosyltransferase, UDP-Gal
- ▼ 3. CMP-SA-PEG, ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

#### FIG. 37D

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

- Sialidase
   CMP-SA-PEG (16 mol eq), ST3Gal3
   CMP-SA, ST3Gal3
- a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG.

### FIG. 37E

## 134/498

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

1. CMP-SA-levulinate, ST3Gal3, 2. H<sub>4</sub>N<sub>2</sub>-PEG

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1;  $R \doteq PEG$ .

### FIG. 37F

```
CHO, BHK, 293 cells, Vero expressed Interferon gamma.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.
```

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

#### FIG. 37G



$$\mathbf{A} \leftarrow \begin{bmatrix} \text{Fuc})_i \\ \text{GlcNAc-GlcNAc-Man} \\ \text{R'})_n \end{bmatrix} \begin{bmatrix} \text{[GlcNAc-(Gal)_a]_s- (Sia)_j - (R)_v ]_r} \\ \text{[[GlcNAc-(Gal)_b]_r- (Sia)_k - (R)_w ]_s} \\ \text{[[GlcNAc-(Gal)_d]_k - (Sia)_m - (R)_y ]_u} \\ \text{[[GlcNAc-(Gal)_d]_k - (Sia)_m - (R)_y ]_u} \end{bmatrix}_q$$

a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0;  $R = \text{modifying group, mannose, oligo-mannose;} \\ R' = H, glycosyl residue, modifying group, glycoconjugate.$ 

FIG. 37H

Insect or fungi cell expressed IF-gamma. a-d, f, h, j-m, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1.

GNT's 1,2,4,5, UDP-GlcNAc
 Galactosyltransferase, UDP-Gal-PEG

a-i, q-u (independently selected) = 0 or 1; j-m = 0; v-y (independently selected) = 1, when e-h (independently selected) is 1; R = PEG.

#### FIG. 371

Yeast expressed IF-gamma. a-m=0; q-y (independently selected) = 0 to 1; p=1; R (branched or linear) = Man, oligomannose.

- 1. Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.

### FIG. 37J

```
CHO, BHK, 293 cells, Vero expressed IF-gamma. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase.

```
a-m, q-u (independently selected) = 0 or 1;
p = 1; n = 0;
v-y (independently selected) = 0 or 1;
R = linker-transferrin.
```

### FIG. 37K

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1; n, v-y = 0.
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h, p = 1;
n, v-y (independently selected) = 0 or 1;
R = PEG.
```

ST3Gal3

### FIG. 37L

## 138/498

```
Insect or fungi cell expressed IF-gamma.
a-d, f, h, j-n, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.

1. GNT's 1 & 2, UDP-GlcNAc-PEG

a-d, f, h, j-n, s, u, w, y = 0;
e, g, i, r, t, q (independently selected) = 0 or 1;
p = 1; v, x (independently selected) = 1,
when e, g (independently selected) is 1;
R = PEG.
```

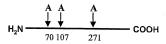
#### FIG. 37M

```
CHO, BHK, 293 cells, Vero expressed
Interferon gamma.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.

1. CMP-SA-PEG, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1;
e-h = 1; j-m (independently selected) = 0-2;
v-y (independently selected) = 1,
when j-m (independently selected) = 2;
R = PEG.
```

### FIG. 37N



$$\mathbf{A} \leftarrow \begin{bmatrix} (\operatorname{Fuc})_{i} & (\operatorname{Gal})_{a} \mathbf{1}_{c}^{-} & (\operatorname{Sia})_{j}^{-} & (\operatorname{R})_{v} \end{bmatrix}_{t}^{T} \\ = (\operatorname{GlcNAc-GlcNAc-Man} & (\operatorname{IGlcNAc-(Gal)}_{a} \mathbf{1}_{f}^{-} & (\operatorname{Sia})_{i}^{-} & (\operatorname{R})_{v} \end{bmatrix}_{t}^{T} \\ = (\operatorname{IGlcNAc-(Gal)}_{a} \mathbf{1}_{g}^{-} & (\operatorname{Sia})_{i}^{-} & (\operatorname{R})_{x} \end{bmatrix}_{t}^{T} \\ = (\operatorname{IGlcNAc-(Gal)}_{a} \mathbf{1}_{g}^{-} & (\operatorname{Sia})_{i}^{-} & (\operatorname{R})_{y} \end{bmatrix}_{t}^{T} \\ = (\operatorname{IGlcNAc-(Gal)}_{a} \mathbf{1}_{g}^{-} & (\operatorname{Sia})_{g}^{-} & (\operatorname{Sia})_{$$

a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 38A

CHO, BHK, 293 cells, Vero or transgenic animal expressed  $\alpha_1$  antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

 Sialidase
 CMP-SA-PEG (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 1, when j-m (independently selected) is 1; R = PEG.

#### FIG. 38B

CHO, BHK, 293 cells, Vero or transgenic animal expressed  $\alpha_1$  antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

 Sialidase
 CMP-SA-PEG (1.2 mol eq), ST3Gal3
 CMP-SA (16 mol eq), ST3Gal3

a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y (independently selected) = 0 or 1; R = PEG

FIG. 38C

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed alpha-1 antitrypsin.

a-d, i-m, q-u (independently selected) = 0 or 1;

e-h = 1; v-y = 0.

1. Sialidase
2. CMP-SA-PEG (16 mol eq),
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

ST3Gal3 3. CMP-SA. ST3Gal3

#### FIG. 38D

```
CHO, BHK, 293 cells, Vero or transgenic animal expressed α<sub>1</sub>-antitrypsin.
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y = 0.
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>4</sub>N<sub>2</sub>-PEG
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = PEG.
```

#### **FIG. 38E**

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CHO, BHK, 293 cells, Vero expressed  $\alpha_1$ -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 38F



$$\mathbf{A} \overset{\text{(Fuc)}_{i}}{\underset{\text{(R')}_{n}}{\text{(Fuc)}_{i}}} \overset{\text{(Fuc)}_{i}}{\underset{\text{(R')}_{n}}{\text{(Fuc)}_{i}}} \overset{\text{(Fuc)}_{i}}{\underset{\text{(IGIeNAc-(Gal)_{a}]_{a}^{-}}{\text{(Sia)}_{b}^{-}}} \overset{\text{(Sia)}_{j}^{-}}{\underset{\text{(R)}_{w}}{\text{(Sia)}_{k}^{-}}} \overset{\text{(R)}_{w}}{\underset{\text{(R')}_{n}}{\text{(Fic)}_{n}}} \overset{\text{(Fuc)}_{i}}{\underset{\text{(IGIeNAc-(Gal)_{a}]_{h}^{-}}{\text{(Sia)}_{m}^{-}}} \overset{\text{(R)}_{w}}{\underset{\text{(R')}_{n}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}}{\underset{\text{(IGIeNAc-(Gal)_{a}]_{h}^{-}}{\text{(Sia)}_{m}^{-}}} \overset{\text{(R)}_{w}}{\underset{\text{(R)}_{y}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}^{-}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}^{-}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{y}}} \overset{\text{(R)}_{w}^{-}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{w}^{-}}} \overset{\text{(R)}_{w}^{-}}{\underset{\text{(R)}_{w}^{-}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{w}^{-}}} \overset{\text{(R)}_{w}^{-}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}_{w}^{-}}} \overset{\text{(R)}_{w}^{-}}{\underset{\text{(R)}_{w}^{-}}{\text{(R)}$$

a-d, i, n, p-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 6.

j-m (independently selected) = 0 to 100.

v-y=0;

R = modifying group, mannose, oligo-mannose;

R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG. 38G

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```
Insect or fungi cell expressed \alpha_1-antitrypsin. a-d, f, h, j-m, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1; j-m = 0; v-y (independently selected) = 1, when e-h (independently selected) is 1; R=PEG.
```

### FIG. 38H

```
Yeast expressed \alpha_1-antitrypsin.

a-m=0; q-y (independently selected) = 0 to 1;

p=1; R (branched or linear) = Man, oligomannose.
```

- Endoglycanase
- 2. Galactosyltransferase, UDP-Gal
- → 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

#### FIG. 381

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CHO, BHK, 293 cells, Vero expressed  $\alpha_1$ -antitrypsin. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

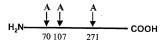
- 1. CMP-SA-linker-Gal-UDP, ST3Gal3
- 2. Galactosyltransferase, transferrin treated with endoglycanase

a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1;

R = linker-transferrin.

FIG. 38J

## 146/498



$$(Fuc)_{i} \\ \mathbf{A} \leftarrow (Glc)_{i} \\ \mathbf{A} \leftarrow (Glc)_{i} \\ (R')_{p} \\ (R')_{q} \\ (R$$

a-d, i, n-u (independently selected) = 0 or 1.

e-h (independently selected) = 0 to 4.

j-m (independently selected) = 0 to 20.

R = polymer;

R', R" (independently selected) = sugar, glycoconjugate.

FIG. 38K

## 147/498

```
Yeast expressed alpha-1 antitrypsin. a-h, i-m, p, q=0; R (independently selected) = mannose, oligomannose; polymannose; r-u, v-y (independently selected) = 0 or 1; n, o = 1.
```

- endoglycanase
- ▼ 2. Galactosyltransferase, UDP-Gal-PEG

```
a-h, i-o, q, r-u, v-y = 0; p = 1.
R" = Gal-PEG.
```

### FIG. 38L

```
Plant expressed alpha-1 antitrypsin. a-d, f, h, j-m, s, u, v-y = 0; e, g, i, q, r, t (independently selected) = 0 or 1; n = 1; R' = xylose
```

- 1. hexosaminidase.
- 2. alpha mannosidase and xylosidase
- 3. GlcNAc transferase, UDP-GlcNAc-PEG

#### FIG. 38M

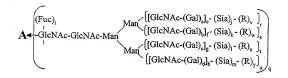
CHO, BHK, 293 cells, Vero, transgenic animal expressed  $\alpha_1$  antitrypsin. a-h, i-o, r-u (independently selected) = 0 or 1; p, q, v-y = 0.

 CMP-SA-PEG, ST3Gal3

a-h, i-o, r-u (independently selected) = 0 or 1; p, q = 0; v-y (independently selected) = 0 or 1; R = PEG.

FIG. 38N





a-d, i, q-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = polymer.

FIG. 39A

# 150/498

```
CHO, BHK, 293 cells, Vero expressed Cerezyme a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 1,
when j-m (independently selected) is 1;
R = PEG.
```

### FIG. 39B

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y=0.
```

- 1. Sialidase
  2. CMP-SA-M-6-P (1.2 mol eq),
  ST3Gal3
  - 3. CMP-SA (16 mol eq), ST3Gal3

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = mannose-6-phosphate
```

#### FIG. 39C

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.
```

```
    Sialidase
    CMP-SA-PEG (16 mol eq),
ST3Gal3
    CMP-SA, ST3Gal3
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = Mannose-6-phosphate
```

#### FIG. 39D

```
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.
```

```
    CMP-SA-levulinate, ST3Gal3,
buffer, salt
    H<sub>4</sub>N,-spacer-M-6-P or clustered M-6-P
```

```
a-d, i-m, q-u (independently selected) = 0 or 1;
e-h = 1; v-y (independently selected) = 0 or 1;
R = M-6-P or clustered M-6-P
```

### FIG. 39E

CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h=1; v-y=0.

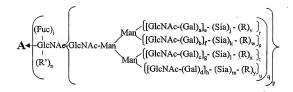
1. CMP-SA, α2,8-ST

a-d, i, q-u (independently selected) = 0 or 1; e-h = 1; j-m (independently selected) = 0-20; v-y (independently selected) = 0.

FIG. 39F

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a-d, i, n, p-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 6. j-m (independently selected) = 0 to 100. v-y = 0; R = modifying group, mannose, oligo-mannose; R' = H, glycosyl residue, modifying group, glycoconjugate.

FIG: 39G

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```
Insect cell expressed Cerezyme.
a-d, f, h, j-m, s, u, v-y = 0;
e, g, i, q, r, t (independently selected) = 0 or 1.
```

```
    GNT's 1,2,4,5, UDP-GlcNAc
    Galactosyltransferase, UDP-Gal-PEG
```

```
a-i, q-u (independently selected) = 0 or 1;

j-m = 0;

v-y (independently selected) = 1,

when e-h (independently selected) is 1;

R = PEG.
```

### FIG. 39H

```
Yeast expressed Cerezyme.

a-m = 0; q-y (independently selected) = 0 to 1;

p = 1; R (branched or linear) = Man, oligomannose.
```

- Endoglycanase
   Galactosyltransferase, UDP-Gal
- 3. CMP-SA-PEG, ST3Gal3

```
a-m, p-y = 0; n (independently selected) = 0 or 1; R' = -Gal-Sia-PEG.
```

### FIG. 391

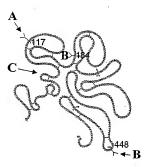
CHO, BHK, 293 cells, Vero expressed Cerezyme. a-d, i-m, q-u (independently selected) = 0 or 1; e-h = 1; v-y = 0.

- 1. CMP-SA-linker-SA-CMP, ST3Gal3
- 2. ST3Gal3, desialylated transferrin.3. CMP-SA, ST3Gal3

a-m, q-u (independently selected) = 0 or 1; p = 1; n = 0; v-y (independently selected) = 0 or 1; R = linker-transferrin.

FIG. 39J

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$$(Fuc)_{i} \\ \textbf{B} \leftarrow GlcNAc - GlcNAc -$$

$$C \longleftarrow \text{-(Fuc)}_{0-1} \qquad A \longleftarrow \text{-GicNAc-GicNAc-Man} \qquad \underbrace{\text{Man-[Man]}_{0-12}}_{\text{Man}} = \underbrace{\text{[Man]}_{0-6}}_{\text{[Man]}_{0-6}}$$

a-d, i, n-u (independently selected) = 0 or 1. e-h (independently selected) = 0 to 4. j-m (independently selected) = 0 to 20. R = polymer; R' = sugar, glycoconjugate.

FIG. 40A

```
CHO, BHK, 293 cells, Vero expressed tPA
a-g, n = 1; h = 1 to 3;
j-m, i, (independently selected) = 0 or 1;
r-u (independently selected) = 0 to 1; o, v-y = 0.
```

```
1. Mannosidase(s), sialidase
2. GNT1,2 (4 and/or 5) UDP-GlcNAc
3. Gal transferase, UDP-Gal
4. CMP-SA-PEG, ST3Gal3
```

```
A = B; a-g, n = 1; h = 1 to 3;
i, r-u (independently selected) = 0 or 1;
o = 0; j-m, v-y (independently selected) = 0 or 1;
R = PEG
```

#### FIG. 40B

```
Insect or fungi cell expressed tPA A=B; a-d, f, h, j-o, s, u, v-y = 0; e, g, i, n, r, t (independently selected) = 0 or 1.
```

```
1. GNT's 1&2, UDP-GlcNAc
2. Galactosyltransferase, UDP-Gal
3. CMP-SA-PEG. ST3Gal3
```

```
\begin{array}{lll} A=B; & b, d, \ f, \ h, \ k, \ m, \ o, \ s, \ u, \ w, \ y=0; \\ a, c, e, \ g, \ i, \ r, \ t \ \ (independently \ selected)=0 \ or \ 1; \\ n=1; \ j, \ l, \ v, \ x \ \ (independently \ selected)=0 \ or \ 1; \\ R=PBG. \end{array}
```

#### FIG. 40C

```
Yeast expressed tPA B = A; i = 0.
```

```
    endoglycanase
    Galactosyltransferase,
    UDP-Gal-PEG
```

UDP-Gal-PEG

```
A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.
```

#### FIG. 40D

```
\begin{split} &\text{Insect or fungi cell expressed tPA} \\ &A=B; \ a\text{-d}, f, h, j\text{-o}, s, u, v\text{-y}=0; \\ &e, g, i, n, r, \ t \ (independently selected)=0 \ or \ 1. \end{split}
```

- 1. alpha and beta mannosidases
- 2. Galactosyltransferase, UDP-Gal-PEG

```
A = B; a-n, r-y = 0; o = 1; R' = Gal-PEG.
```

#### FIG. 40F

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```
Insect or fungi cell expressed tPA A=B;\ a-d,\ f,\ h,\ j-o,\ s,\ u,\ v-y=0;\ e,\ g,\ i,\ n,\ r,\ t\ (independently\ selected)=0\ or\ 1.
```

- 1. GNT's 1&2, UDP-GICNAC
- 2. Galactosyltransferase, UDP-Gal-PEG

#### FIG. 40F

```
Insect or fungi cell expressed tPA A=B;\ a-d,\ f,\ h,\ j-o,\ s,\ u,\ v-y=0; e, g, i, n, r, t (independently selected) = 0 or 1.
```

- GNT's 1 & 2, UDP-GlcNAc
   Galactosidase (synthetic enzym)
- Galactosidase (synthetic enzyme),
   PEG-Gal-F.

```
A = B; b, d, f, h, j-o, s, u, w, y = 0;
a, c, e, g, i, r, t, v, x (independently selected)= 0 or 1;
n = 1; R = PEG.
```

### FIG. 40G